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Research Article

Comparative acute toxicity of chlorofet and thiodan to the amphipod Parhyale basrensis (Salman, 1986) from Iraq

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Abstract

The pesticides chlorofet (active ingredient chlorpyrifos) and thiodan (active ingredient endosulfan) are widely used and released to surface waters in Iraq. To determine the potential effects of these pesticides on the aquatic fauna of Iraq, we tested the acute toxicity of chlorofet and thiodan using the talitrid amphipod *Parhyale basrensis*. Our results indicate that *P. basrensis* is more sensitive to chlorofet than thiodan. When expressed in terms of the active ingredients, *P. basrensis* is the most sensitive amphipod species that has been tested to date to endosulfan. In contrast, *P. basrensis* appears to be of average sensitivity to chlorpyrifos.

Keywords: Acute toxicity; chlorofet; thiodan; chlorpyrifos; endosulfan; *Parhyale basrensi*

Introduction

In Iraq, pesticides are used for domestic and agricultural pest control as they are elsewhere in the world, but they are also used to catch fish and birds. To catch fish, fishermen pour pesticides directly into a water body and then collect the fish when they float to surface. The fish collected are then either sold as food or made into chicken feed. Some fishermen also mix pesticides with bait to catch birds during their migration from Europe to the southern Iraqi marshes, which are also sold as food. Due to agricultural runoff, overspray, chemical bird baiting, and especially chemical fishing (which should result in greater releases than the other uses), aquatic organisms in Iraq may be exposed to significant quantities of pesticides.

Two of the most commonly used pesticides in Iraq are chlorofet (active ingredient chlorpyrifos) and thiodan (active ingredient endosulfan) (Al-Hilffi, 2005). According to the Canadian Council of Ministers of the Environment (CCME, 2008, CCME, 2010), amphipods are among the most sensitive aquatic species to acute chlorpyrifos toxicity and among the most sensitive aquatic invertebrates to acute endosulfan toxicity. Additionally, amphipods are ecologically important as they are abundant in the marshes of southern Iraq and are the principle prey of many birds, fish, and larger invertebrates (Naser, 2005). Here, we report the results of acute chlorofet and thiodan toxicity tests using the amphipod *Parhyale basrensis*.

Materials and Methods

Amphipods were collected from the river Garmmat Ali (30°34'16" N, 47°44'59" E) in July 2011 and identified according to (Salman, 1986). The average length of the amphipods used in the experiment was 6.8 mm (±standard deviation of 0.1 mm), with a sex ratio of 1 female: 1.3 males. Thirty amphipods were maintained per beaker in 10 L beakers filled with dechlorinated tap water with continuous aeration (pH 7.6, dissolved oxygen 7.5 mg/l, salinity 0.9 practical salinity units, total hardness 137 mg/l as CaCO3) and a 12 L: 12 D photoperiod for 7 days prior to the start the experiment.

Static acute bioassays were conducted using commercial grade Chlorofet-48 TC® (48% chlorpyrifos, other contents unknown; made in Jordan) and Thiodan-35 EC® (35% endosulfan, other contents unknown; made in Iran). The nominal concentrations used were 0, 0.2, 0.3, 0.4, 0.5, 0.6, and 0.7 µg/L for chlorofet and 0, 0.45, 0.55, 0.65, 0.75, 0.85, 0.95, and 0.95 µg/L for thiodan. Ten amphipods were tested at each concentration, with each amphipod kept in a separate 500 mL beaker.

Every three hours for 96 hours, each amphipod was gently prodded under a dissecting microscope with a needle. Amphipods that did not respond within one minute were recorded as dead and were removed from the experiment.

The concentrations of chlorpyrifos and endosulfan in chlorofet and thiodan, respectively, were calculated by multiplying the concentration of chlorpyrifos and endosulfan used in each treatment by the percent of the active ingredient in the formulation.

Differences in length among treatments were evaluated using a Kruskall-Wallis test in XLSTAT 2011.4.01. The Cochran Armitrage trend test was performed using USEPA's (BMDS, 201a) BMDS v2.2. The gamma, logistic, multistage, probit, quantal-linear, and Weibull models were fit using BMDS and the Gompertz model was fit using XLSTAT. Since BMDS does not calculate pseudo-r2 values, the McFadden's pseudo-r2 was calculated, as recommended by (Menarad, 2000), using the following formula:

$$R_L^2 = 1 - \frac{\text{LogLikelihood fitted model}}{\text{LogLikelihood intercept only}}$$

Note that the loglikelihood of the a) fitted model is also referred to as the "model with predictors" and b) that the loglikelihood of the intercept only is also referred to as the "reduced model" or the "model without predictors."

Results and discussion

There was no difference in amphipod lengths among the treatments for both chlorofet (Kruskall-Wallis test, K = 1.790, d.f. 5, p = 0.88) and thiodan (Kruskall-Wallis test, K = 1.37, d.f. = 6, p = 0.97). There was also no difference in mortality between males and females within each treatment for both chlorofet and thiodan (z-test, p > 0.1 for all doses). Therefore, the data for males and females was combined in the analyses below. No mortality was observed in the controls for both pesticide formulations tested.

Prior to fitting dose-response models to the data, we verified that there was a significant dose-related effect of both chlorofet (Cochran Armitrage trend test, z = 7.50, p <0.0001) and thiodan (Cochran Armitrage trend test, z = 8.11, p <0.0001) on survival. For both chlorofet (Figure 1) and chlorpyrifos, the best fitting multistage model had a better fit, as determined using Akike's Information Criterion (AIC), to the data (AIC = 123.2, pseudo-r2 = 0.37, Log(likelihood) = -94.22, p < 0.0001; goodness of fit chi-square = 2.23, d.f = 5, p = 0.82) than the other models. The equations for the best fitting multistage models are as follows: a) chlorofet $\%\mathrm{Mortality} = 1 - \mathrm{e}^{(-1.10\times\mathrm{C})-(4.56\times\mathrm{C}^2)}$ and b) chlorpyrifos $\%\mathrm{Mortality} = 1 - \mathrm{e}^{(-2.30\times\mathrm{C})-(19.79\times\mathrm{C}^2)}$, where C is the concentration in µg/L.

For both thiodan (Figure 2) and endosuflan, the best fitting multistage model had a better fit to the data (AIC = 132.4, pseudo-r2 = 0.37, Log(likelihood) = -104.20, p < 0.0001; goodness of fit chi-square = 4.62, d.f = 7, p = 0.71) than the other models. The equations for the best fitting multistage models are as follows: a) thiodan $\%\mathrm{Mortality} = 1 - \mathrm{e}^{-2.78\times\mathrm{C}^2}$ and b) endosulfan $\%\mathrm{Mortality} = 1 - \mathrm{e}^{-22.69\times\mathrm{C}^2}$.

The amphipod *P. basrensis* was much more sensitive to chlorofet (the concentration lethal to 50% of the test organisms (LC50) was 0.29 µg/L) than to thiodan (LC50 0.50 µg/L). However, when calculated in terms of the active ingredients, the difference was not as pronounced; i.e., the LC50 for chlorpyrifos was 0.14 µg/L whereas the LC50 for endosulfan was 0.17 µg/L (Table 1 &2).

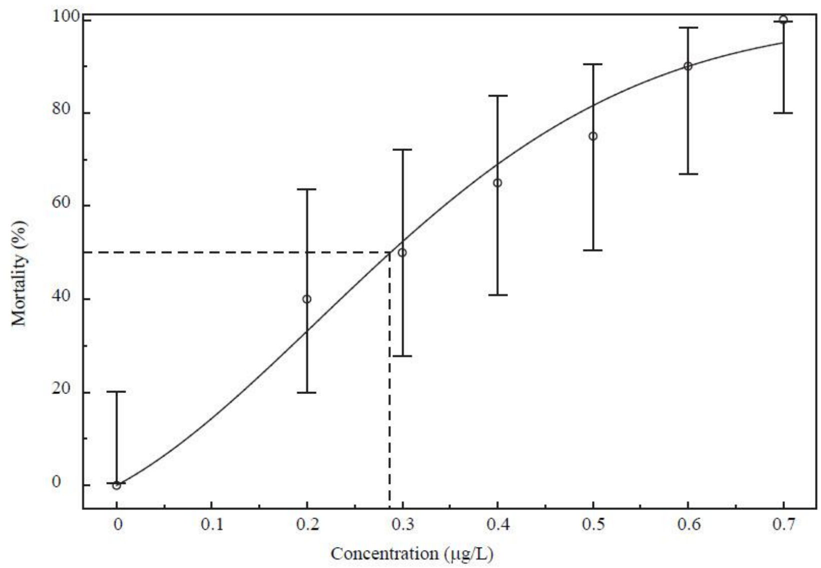

Figure 1. Mortality of the amphipod *P. basrensis* when exposed to chlorofet for 96 hours. Open circles represent the observed mortality rate, with the error bars showing the 95% confidence interval on the mortality rate. Line connecting the open circles is the best fitting multistage model. Dashed line represents the calculated LC50.

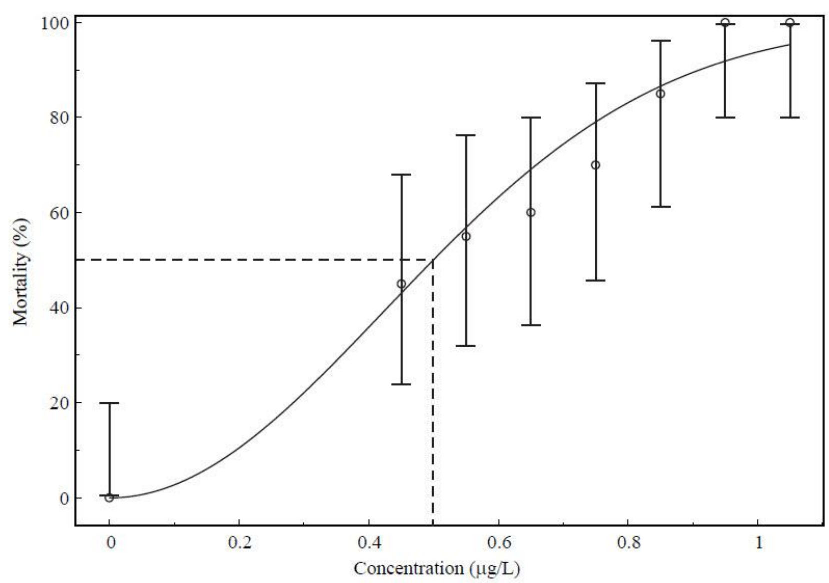

Figure 2. Mortality of the amphipod *P. basrensis* when exposed to thiodan for 96 hours. Open circles represent the observed mortality rate, with the error bars showing the 95% confidence interval on the mortality rate. Line connecting the open circles is the best fitting multistage model. Dashed line represents the calculated LC50.

Table 1. 96hr acute toxicity of chlorfet and chlorpyrifos to the amphipod *P. basrensis*.

Lethal Concentration (%)	Chlorofet		Chlorpyrifos	
	Concentration (µg/L)	95% Confidence Limits	Concentration (µg/L)	95% Confidence Limits
5	0.040	0.018 – 0.091	0.019	0.009 – 0.044
50	0.29	0.22 – 0.34	0.14	0.11 – 0.16
95	0.70	0.60 – 0.91	0.34	0.29 – 0.43

Table 2. 96hr acute toxicity of thiodan and endosulfan to the amphipod *P. basrensis*.

Lethal Concentration (%)	Thiodan		Endosulfan	
	Concentration (µg/L)	95% Confidence Limits	Concentration (µg/L)	95% Confidence Limits
5	0.14	0.055 – 0.15	0.048	0.019 – 0.052
50	0.50	0.43 – 0.55	0.17	0.15 – 0.19
95	1.0	0.95 – 1.2	0.36	0.33 – 0.41

To evaluate whether *P. basrensis* is more sensitive to chlorpyrifos and endosulfan than other amphipods, we compared the LC50s calculated here to the results of other 96hr acute toxicity tests listed in (United States Environmental Protection Agency, 2011b) ECOTOX database (Table 3). *Parhyale basrensis* appears to be just as sensitive as most other amphipods to chlorpyrifos (i.e., LC50 of 0.14 vs. 0.04 to 2.9 µg/L; Table 3). According to (United States Environmental Protection Agency, 2011b), *P. basrensis* appears to be an order of magnitude more sensitive to endosulfan than other amphipods (LC50 of 0.17 vs. 5.7 to 6.0 µg/L; Table 3). While the endosulfan LC50s reported by (United States Environmental Protection Agency, 2011b) are approximately an order of magnitude higher than what was calculated here, (Leight, and van Dolah, 1999) reported 96hr LC50s for the amphipod *Gammarus palustris* exposed to endosulfan of 0.43 and 0.50 µg/L, depending upon whether static or renewal tests were performed, respectively. Thus, *P. basrensis* is appears to be slightly more sensitive than other amphipods tested to date to endosulfan.

Among the other species from Iraqi marshes that have been tested so far, P. basrensis (LC50 0.5 µg/L) is more sensitive to thiodan than the shrimp *Caridina babaulti basrensis* LC50 2 µg/L (Naser, 2010) and the freshwater snail *Lymnaea radix cor* adult (LC50 910 µg/L, immature LC50 380 µg/L) (Yasser et al., 2008). *Parhyale basrensis* is also more sensitive to chlorofet than the freshwater snail *Bellamya bengalensis* (LC50 1,232 µg/L) (Yasser et al., 2010). Although the freshwater fishes of Iraq have not been tested, fishes are generally more than one order of magnitude less sensitive to chlorpyrifos than amphipods (CCME, 2008) but approximately one order of magnitude more sensitive than amhipods to endosulfan (CCME, 2010). This indicates that chemical fishing using chlorofet is much more likely to adversely impact amphipods than thiodan; i.e., the concentration of chlorpyrifos necessary to cause fish to rise to the surface is likely to be much higher than that necessary to kill amphipods, whereas the concentration of endosulfan necessary to cause fish to rise to the surface is likely to be lower than the concentration that would kill amphipods.

Table 3. Freshwater amphipod 96hr LC50s of chlorpyrifos and endosulfan as reported by USEPA (2011b) and ranked by LC50.

Chlorpyrifos		Endosulfan	
Species	Concentration (µg/L)	Species	Concentration (µg/L)
Hyalella azteca[a]	0.04 – 0.14	*Parhyale basrensis*[b]	0.17
Gammarus pulex	0.07	*Gammarus palustris*[c]	0.43 – 0.54
Gammarus lacustris	0.11	*Hyalella azteca*[d]	5.7
Parhyale basrensis[b]	0.14	*Gammarus lacustris*	5.8
Gammarus pseudolimnaeus	0.18	*Gammarus fasciatus*	6.0
Gammarus fasciatus	0.32		
Gammarus fossarum	2.9		

Notes**:**

a – Results of four separate studies.

b - This study.

c – Leight and Dolah (1999), not included in USEPA (2011b).

d – For (α+β)-endosulfan

From this study, it is evident that *P. basrensis* is just as sensitive to chlorpyrifos as other amphipods but appears to be more sensitive than other amphipods to endosulfan. The amphipod *P. basrensis* is also the most sensitive aquatic species tested to date from the marshes in Iraq to both chlorofet and thiodan. Chemical fishing using endosulfan containing pesticides (e.g., thiodan) is less likely to adversely affect amphipods than chemical fishing using chlorpyrifos containing pesticides (e.g., chlorofet) are used.

Conclusion

From this study, it is evident that *P. basrensis* is just as sensitive to chlorpyrifos as other amphipods but appears to be more sensitive than other amphipods to endosulfan. The amphipod *P. basrensis* is also the most sensitive aquatic species tested to date from the marshes in Iraq to both chlorofet and thiodan. Chemical fishing using endosulfan containing pesticides (e.g., thiodan) is less likely to adversely affect amphipods than chemical fishing using chlorpyrifos containing pesticides (e.g., chlorofet) are used.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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